

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

Helmut SCHWAB et al. : Attn: APPLICATION BRANCH

Serial No. [NEW] : Docket No. 2001-1882A

Filed January 16, 2002 :

NEW GENES CONTAINING A DNA
SEQUENCE CODING FOR HYDROXY-
NITRILE LYASE, RECOMBINANT
PROTEINS DERIVED THEREFROM AND
HAVING HYDROXYNITRILE LYASE
ACTIVITY, AND USE THEREOF :

THE COMMISSIONER IS AUTHORIZED
TO CHARGE ANY DEFICIENCY IN THE
FEE FOR THIS PAPER TO DEPOSIT
ACCOUNT NO. 23-0975.

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents,
Washington, DC 20231

Sir:

In the interest of compact prosecution and to reduce PTO filing fees, please amend the present application as follows:

IN THE CLAIMS:

Please cancel claims 12 and 16 without prejudice to the subject matter thereof.

Please amend claims 7, 11, 13 to 15, and 17 as follows:

7. (Amended) A recombinant protein, which can be prepared in suitable host cells by heterologous expression of the DNA sequence of the *Prunus amygdalus HNL* genes as claimed in claim 1.

11. (Amended) The recombinant protein as claimed in claim 7, wherein the protein has the amino acid sequence derived from the nucleotide sequence of the gene containing a DNA

sequence coding for hydroxynitrile lyase, which gene can be prepared from a primer combination based on the DNA sequence of the 5'-region of the *Prunus serotina* *mdl5* gene and of the *Prunus amygdalus* *MDL1* gene, subsequent amplification with a DNA polymerase from organisms containing genes coding for hydronitrile lyase as templates and cloning, and which gene has the nucleotide sequence depicted in figure 1 or is at least 80% identical thereto.

13. **(Amended)** A fusion protein or heterologous protein with hydroxynitrile lyase activity which can be prepared by using a DNA sequence of genes as claimed in claim 1, which codes for the signal peptide of a hydroxynitrile lyase of Rosacea species, and by secretory expression thereof in host cells.

14. **(Amended)** The fusion protein as claimed in claim 13, wherein the fusion protein has the nucleic acid sequence depicted in figure 4, comprising sequences of the gene containing a DNA sequence coding for hydroxynitrile lyase, which gene can be prepared from a primer combination based on the DNA sequence of the 5'-region of the *Prunus serotina* *mdl5* gene and of the *Prunus amygdalus* *MDL1* gene, subsequent amplification with a DNA polymerase from organisms containing genes coding for hydronitrile lyase, as templates and cloning, and which gene has the nucleotide sequence depicted in figure 1 or is at least 80% identical thereto and the *Aspergillus niger* glucose oxidase gene, and also the amino acid sequence according to figure 5, which is derived from said nucleic acid sequence.

17. **(Amended)** A process for preparing (R)- or (S)-cyanohydrins, which comprises reacting aliphatic, aromatic or heteroaromatic aldehydes and ketones with proteins as claimed in claim 7 in an organic, aqueous or 2-phase system or in emulsion in the presence of a cyanide group donor.

Please add the following new claims:

18. (New) The recombinant protein as claimed in claim 7, wherein the protein has the amino acid sequence derived from the nucleotide sequence of the gene containing containing a DNA sequence coding for hydroxynitrile lyase, which gene can be prepared from primers based on the DNA sequence of the 5'-region of the *Prunus serotina* *mdlI* gene, subsequent amplification with a DNA polymerase from organisms containing genes coding for hydronitrile lyase, as templates and cloning, and which has the nucleotide sequence depicted in figure 8 or is at least 80% identical thereto.

19. (New) A process for preparing (R)- or (S)-cyanohydrins, which comprises reacting aliphatic, aromatic or heteroaromatic aldehydes and ketones with proteins as claimed in claim 14 in an organic, aqueous or 2-phase system or in emulsion in the presence of a cyanide group donor.

REMARKS

The above amendment is presented to eliminate multiple dependent claims, thereby reducing PTO filing fees.

Attached hereto is a marked-up version of the changes made to the claims by the current amendment. The attached page is entitled "**Version with Markings to Show Changes Made**".

In the above amendment, original claim 11 is divided into claim 11 (amended) and new claim 18.

Use claims 12 and 16 have been deleted.

Claim 17 has been divided into claim 17 (amended) and new claim 18.

Favorable action on the merits is now requested.

Respectfully submitted,

Helmut SCHWAB et al.

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January 16, 2002

VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE CLAIMS:

Please amend claims 7, 11 to 15, 16 and 17 as follows:

7. (Amended) A recombinant protein, which can be prepared in suitable host cells by heterologous expression of the DNA sequence of the *Prunus amygdalus HNL* genes as claimed in [any of claims] claim 1 [to 6].

11. (Amended) The recombinant protein as claimed in claim 7, wherein the protein has the amino acid sequence derived from the nucleotide sequence of the gene [as claimed in claim 3 or 4] containing a DNA sequence coding for hydroxynitrile lyase, which gene can be prepared from a primer combination based on the DNA sequences of the 5'-region of the *Prunus serotina mdl5* gene and of the *Prunus amygdalus MDL1* gene, subsequent amplification with a DNA polymerase from organisms containing genes coding for hydroxynitrile lyase, as templates and cloning, and which gene has the nucleotide sequence depicted in figure 1 or is at least 80% identical thereto.

13. (Amended) A fusion protein or heterologous protein with hydroxynitrile lyase activity which can be prepared by using a DNA sequence of genes as claimed in claim 1 [to 6], which codes for the signal peptide of a hydroxynitrile lyase of Rosacea species, and by secretory expression thereof in host cells.

14. (Amended) The fusion protein as claimed in claim 13, wherein the fusion protein has the nucleic acid sequence depicted in figure 4, comprising sequences of the gene[as claimed in claim 3] containing a DNA sequence coding for hydroxynitrile lyase, which gene can be prepared from a primer combination based on the DNA sequence of the 5'-region of the *Prunus serotina mdl5* gene and of the *Prunus amygdalus MDL1* gene, subsequent amplification with a DNA polymerase from organisms containing genes coding for hydroxynitrile lyase, as templates and cloning, and which gene has the nucleotide sequence

depicted in figure 1 or is at least 80% identical thereto and the *Aspergillus niger* glucose oxidase gene, and also the amino acid sequence according to figure 5, which is derived from said nucleic acid sequence.

17. (Amended) A process for preparing (R)- or (S)-cyanohydrins, which comprises reacting aliphatic, aromatic or heteroaromatic aldehydes and ketones with proteins as claimed in [any of claims 7-11 or 13-15] claim 7 in an organic, aqueous or 2-phase system or in emulsion in the presence of a cyanide group donor.